

Endometriosis is not associated with or linked to the GALT gene

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Objective: To investigate a possible association between the carrier frequency of the N314D mutation in the galactose-1-phosphate uridyl transferase (GALT) gene and endometriosis and linkage to the short arm of chromosome 9, where the GALT gene resides.

Design: Association and linkage study.

Setting: Population material collected for case and family studies in endometriosis.

Patient(s): Women diagnosed with endometriosis by laparotomy or laparoscopy.

Intervention(s): Association with the GALT gene investigated by genotyping 85 affected women and 213 unrelated control women and a scan for linkage to chromosome 9 in 205 women from 64 families with endometriosis.

Main Outcome Measure(s): Multipoint parametric lod scores and frequency of alleles.

Result(s): There was no significant difference in allele frequency for the N314D polymorphism in patients compared with control subjects. No evidence for linkage was found to chromosome 9p, where the GALT gene resides.

Conclusion(s): The experiments reported herein provide no evidence supporting involvement of the GALT locus in the development of endometriosis. (Fertil Steril® 2001;76:1019–22. ©2001 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, N314D polymorphism, Galactose-1-phosphate uridyl transferase, association, linkage

Increasing evidence supports a genetic component in endometriosis. Studies of twins (1, 2) have suggested that endometriosis may have a genetic basis, including a recent study on 3,096 twin sister pairs, 215 of whom reported endometriosis, a prevalence of 7% (3); concordance in monozygotic twins exceeded that in dizygotic twins by a factor of 2. Furthermore, a number of studies have demonstrated a 3.9- to 6.2-fold increased risk for mothers and a 3.8- to 8.1-fold increased risk for sisters of probands for developing the disease (4–7). Therefore, genetic susceptibility is likely in endometriosis.

Strategies for finding these genes include candidate gene approaches, differential expression studies in endometriotic lesions and eutopic endometrium, or genome-wide scans for linkage. Although genome-wide scans for endometriosis genes are currently being con-

ducted, none has yet been published. Because endometriosis skips generations and exhibits no clear Mendelian pattern of inheritance, multiple genes may interact with several as yet unknown environmental factors to cause the disease.

It has been postulated that endometriosis could be associated with a specific mutation in the gene encoding for galactose-1-phosphate uridyl transferase (GALT), the N314D mutation (8). This mutation is common, with carrier frequencies of 14%–18% in different populations (8–10). The mutation, an A to G transition, replaces asparagine-314 by aspartic acid (11) and has been associated with the Duarte phenotype form of galactosemia (12) and possibly with vaginal agenesis (13). Genetically caused errors of galactose metabolism could then lead to abnormal intrauterine exposure to

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galactose and in the offspring to a defect in canalization of the cervix, with cervical stenosis and increased risk of retrograde menstruation and subsequent endometriosis as a result (13).

The aim of this study was to investigate a possible association between the carrier frequency of the N314D mutation in the GALT gene and endometriosis and to investigate if there is linkage to the short arm of chromosome 9 where the GALT gene resides.

MATERIALS AND METHODS

Sample Selection

Women with pelvic endometriosis diagnosed by laparoscopy and laparotomy from 1981 to 1993 in Iceland were identified by searching a national computerized database of hospital admissions for the relevant International Classification of Diseases (ICD) 9 diagnostic codes, 617.1–617.9. Individual operation notes were examined for each patient to confirm the diagnosis, stage of disease, and severity and to obtain histological confirmation, where available. Disease severity was assessed using the Revised American Fertility Society (1985) classification system stages I–IV (14). All women received an explanatory letter with a questionnaire on symptoms, signs, disease progress, and family history and a consent form asking for their participation. A total of 744 women had medical records, and 491 consented to participate and returned the questionnaire.

The genealogy database at deCODE Genetics was used to identify family connections. This database stores all available genealogy for the last 10 centuries in Iceland, including genealogy manuscripts, censuses, and church books. The genealogy database used for disease projects is encrypted by the Icelandic Data Protection Committee in the same way as the national identity numbers that are used for patient identification (15). Pedigrees were drawn allowing for up to six meiotic events between affected individuals in a family. The genealogy database has been described in detail (16).

To investigate linkage between endometriosis and the GALT gene, blood samples were obtained from 205 affected women with a verified family history of endometriosis and from their relatives. These 205 women belonged to 64 families and the smallest pedigree included two and the largest 13 affected women. The relationships between affected individuals in the families ranged from sister pairs to second cousins.

To investigate an association between endometriosis and the GALT N314D mutation, 85 affected women were selected from among all women in the families described above, preferably those women with more severe disease. Eighty were diagnosed with severe/extensive disease (stages III and IV) and five had minor/mild disease (stages I and II). None of these women were related to any other woman in this group within six meiotic events. Samples from a control

group of 213 unrelated (not related within six meiotic events) Icelandic volunteers of both sexes were also collected.

The study was approved by the University Hospital Ethical Committee (Institutional Review Board) and the Icelandic Data Protection Committee.

Genotyping and Linkage Analysis

DNA was extracted from peripheral blood by an organic extraction method. Forty-seven fluorescently labeled microsatellite markers were used to genotype members from the 64 families for chromosome 9. Polymerase chain reaction (PCR) fragments were separated by electrophoresis on ABI 377 sequencers, and the deCODE GT software (17) was used for allele calling. Multipoint, nonparametric linkage analysis was performed with the Allegro software (18) using the exponential model of Kong and Cox (19).

Sequencing

The N314D locus was sequenced in both directions. The following primers were used in a PCR to amplify a 537-bp segment including the target locus: (5') AGGAGCAAGC-CTTGAAAC and (3') AACTTTGGCAGTCCCTTCT. Amplification reactions were performed on 10 ng of template DNA in a 20- μ L volume by use of AmpliTaq Gold polymerase (PE Biosystems, Foster City, CA). The cycle profile started with 95°C for 12 minutes, followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. Samples were sequenced by use of a BigDye Terminator Cycle Sequencing kit (PE Biosystems). The same primers were used for the cycle sequencing reactions as for the initial amplification step. The cycle sequencing profile was 30 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. Allele frequencies for the GALT N317D polymorphism were calculated in patients and controls.

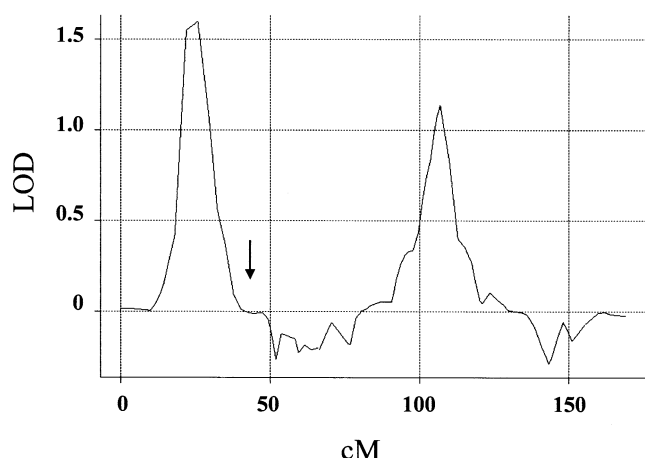
RESULTS

The multipoint nonparametric lod scores for chromosome 9 are shown in Figure 1. The 64 families were genotyped using 47 polymorphic microsatellite markers for chromosome 9. Although small but positive linkage was found to chromosome 9 (lod scores of 1.1 and 1.6), they were not at the GALT gene locus. Therefore, no evidence of linkage to the GALT region was found.

Of the 85 unrelated women with endometriosis, 13 (15.3%) were heterozygous (NM) for the N314D polymorphism compared with 43 (20.2%) of 213 controls. The carrier frequency of the polymorphism in the unrelated cases was not significantly different from the control frequency (Table 1). None of the unrelated cases was homozygous for the N314D polymorphism (MM), whereas two were found in the control group. Men and women in the control group had similar carrier frequencies, 20.4% and 20.0%, respectively.

FIGURE 1

Multipoint nonparametric linkage analysis for chromosome 9 showing no evidence of linkage to the GALT locus (site indicated by an arrow).



Stefansson. Nonassociation of endometriosis with GALT gene. *Fertil Steril* 2001.

No other polymorphism was detected in exon 10 of the GALT gene.

DISCUSSION

Endometriosis is a common disorder whose etiology and pathogenesis are poorly understood. A few genes have been implicated in the disease based on association and functional studies. One of these candidate genes is the GALT gene. The disease has been proposed to be associated with inborn errors of galactose metabolism, in particular with a specific mutation, N314D in the GALT gene.

The GALT gene is an interesting candidate gene for endometriosis under the supposition that there is a connection among endometriosis, galactose metabolism errors, and

vaginal agenesis or cervical canal narrowing (13, 20). A number of variants have been found in the GALT gene that have been shown to be functional at the level of transcription and enzyme activity and that are therefore biologically relevant. More than 100 different base changes have been documented along the length of the GALT gene (21). These include missense, nonsense, and silent mutations, insertions and deletions, splice site mutations, and single base changes deep in introns. The “coding” mutations most widely distributed are Q188R, K285N, and N314D.

The carrier frequency of the GALT polymorphism N314D has been reported to be 14%–18% in a number of studies (8–10). Association was initially reported between the N314D polymorphism and endometriosis by Cramer et al. (8) in a US population, i.e., 30% in cases and 14% in controls. In our control group, the carrier frequency was 20.2%, which is similar to what is reported for other Caucasian control populations (9, 10). Since the difference between cases and controls was not significant, the N314D polymorphism is unlikely to influence the risk of developing endometriosis in Icelandic women. The chromosome 9 scan on the 205 affected women from 64 families did not show any evidence of linkage to chromosome 9p, where the GALT gene is located.

Since the original report of Cramer et al. (8), a few groups have investigated the N314D polymorphism and the proposed association with endometriosis. Morland et al. (9) found no association with endometriosis. Hadfield et al. (10) investigated the N314D polymorphism in patients with endometriosis and controls in a UK population. They fractionated the group of affected women into subgroups of moderate to severe disease and sporadic or familial cases but did not find any difference in frequency of the N314D polymorphism between investigated groups. Based on our results and existing reports, it is not likely that the gene encoding for the GALT protein or a gene in its vicinity is associated with endometriosis.

TABLE 1

Distribution and frequencies of the N314D alleles in Icelandic patients with endometriosis and control groups.

Group	Total	NN frequency		NM frequency		MM frequency		N314D allele
		n	%	n	%	n	%	
Endometriosis	85	72	84.7 (77.0–92.3)	13	15.3 (7.7–22.9)	0	0 (0)	7.6 (3.6–11.6)
Control, all	213	168	78.8 (73.4–84.3)	43	20.2 (14.9–25.5)	2	0.9 (0–2.2)	11.0 (8.1–13.9)
Control, men	103	80	77.7 (69.7–85.7)	21	20.4 (12.6–28.2)	2	1.9 (0–4.61)	12.1 (7.64–16.6)
Control, women	110	88	80.0 (72.5–87.5)	22	20.0 (12.5–27.5)	0	0 (0)	10.0 (6.0–14.0)

Note: Numbers in parentheses are 95% confidence intervals. MM = homozygous individuals; NM = heterozygous individuals; NN = normal genotypes (no mutation).

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References

1. Moen MH. Endometriosis in monozygotic twins. *Acta Obstet Gynecol Scand* 1994;73:59–62.
2. Hadfield RM, Mardon HJ, Barlow DH, Kennedy SH. Endometriosis in monozygotic twins. *Fertil Steril* 1997;68:941–2.
3. Treloar SA, O'Connor DT, O'Connor VM, Martin NG. Genetic influences on endometriosis in an Australian twin sample. *Fertil Steril* 1999;71:701–10.
4. Simpson JL, Elias S, Malinak LR, Buttram VC. Heritable aspects of endometriosis. I. Genetic studies. *Am J Obstet Gynecol* 1980;137:327–31.
5. Lamb K, Hoffmann RG, Nichols TR. Family trait analysis: a case-control study of 43 women with endometriosis and their best friends. *Am J Obstet Gynecol* 1986;154:596–601.
6. Moen MH, Magnus P. The familial risk of endometriosis. *Acta Obstet Gynecol Scand* 1993;72:560–4.
7. Coxhead D, Thomas EJ. Familial inheritance of endometriosis in a British population. A case control study. *J Obstet Gynaecol* 1993;13:42–4.
8. Cramer DW, Hornstein MD, Ng WG, Barbieri RL. Endometriosis associated with the N314D mutation of galactose-1-phosphate uridyl transferase (GALT). *Mol Hum Reprod* 1996;2:149–52.
9. Morland S, Morland SJ, Jiang X, Hitchcock A, Thomas EJ, Campbell IG. Mutation of galactose-1-phosphate uridyl transferase and its association with ovarian cancer and endometriosis. *Int J Cancer* 1998;77:825–7.
10. Hadfield RM, Manek S, Nakago S, Mukherjee S, Weeks DE, Mardon HJ, et al. Absence of a relationship between endometriosis and the N314D polymorphism of galactose-1-phosphate uridyl transferase in a UK population. *Mol Hum Reprod* 1999;5:990–3.
11. Reichardt JK, Woo SL. Molecular basis of galactosemia: mutations and polymorphisms in the gene encoding human galactose-1-phosphate uridyl transferase. *Proc Natl Acad Sci USA* 1991;88:2633–7.
12. Elsas LJ, Dembure PP, Langley S, Paulk EM, Hjelm LN, Fridovich-Keil J. A common mutation associated with the Duarte galactosemia allele. *Am J Hum Genet* 1994;54:1030–6.
13. Cramer DW, Goldstein DP, Fraer C, Reichardt JK. Vaginal agenesis (Mayer-Rokitansky-Kuster-Hauser Syndrome) associated with the N314D mutation of galactose-1-phosphate uridyl transferase (GALT). *Mol Hum Reprod* 1996;2:145–8.
14. The American Fertility Society. Revised American Fertility Society Classification of Endometriosis: 1985. *Fertil Steril* 1985;43:351–2.
15. Gulcher J, Kristjansson K, Gudbjartsson H, Stefansson K. Protection of privacy by third-party encryption in genetic research in Iceland. *Eur J Hum Genet* 2000;8:739–42.
16. Gudmundsson H, Gudbjartsson DF, Kong A, Gudbjartsson H, Frigge M, Gulcher JR, et al. Inheritance of human longevity in Iceland. *Eur J Hum Genet* 2000;8:743–9.
17. Palsson B, Palsson F, Perlin M, Gudbjartsson H, Stefansson K, Gulcher J. Using quality measures to facilitate allele calling in high-throughput genotyping. *Genome Res* 1999;9:1002–12.
18. Gudbjartsson DF, Jonasson K, Frigge ML, Kong A, Allegro, a new computer program for multipoint linkage analysis. *Nat Genet* 2000;25:12–3.
19. Kong A, Cox NJ. Allele-sharing models: LOD scores and accurate linkage tests. *Am J Hum Genet* 1997;61:1179–88.
20. Cramer DW, Ravnkar VA, Craighill M, Ng WG, Goldstein DP, Reilly R. Mullerian aplasia associated with maternal deficiency of galactose-1-phosphate uridyl transferase. *Fertil Steril* 1987;47:930–4.
21. Tyfield L, Reichardt J, Fridovich-Keil J, Croke DT, Elsas LJ II, Strobl W, et al. Classical galactosemia and mutations at the galactose-1-phosphate uridyl transferase (GALT) gene. *Hum Mutat* 1999;13: 417–30.